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Muscarinic M_1 receptor activation reduces maximum upstroke velocity of action potential in mouse right atria

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Abstract

We investigated whether acetylcholine affects cardiac action potentials through the muscarinic M_1 in addition to M_2 receptors in spontaneously beating mouse isolated right atria. A conventional glass microelectrode technique was used for the purpose. Acetylcholine (3–10 μM) reduced the maximum upstroke velocity of the action potentials (V_{max}), followed by an increase. It shortened action potential duration at 90% repolarization, hyperpolarized the resting membrane and decreased the rate of beating. Atropine (3–100 nM) concentration dependently antagonized these effects of acetylcholine. Pirenzepine (10 and 30 nM), a selective muscarinic M_1 receptor antagonist, antagonized acetylcholine (5 μM)-induced reduction of V_{max} without affecting other effects of acetylcholine. In addition, pirenzepine (30 nM) induced an immediate and linear acceleration of the V_{max} reduced by acetylcholine. In contrast, AF-DX 116 (11({2-[(diethylamino)-methyl]-1-piperidyl}acetyl)-5,11-dihydro-6*H*-pyridol[2,3-b][1,4]benzodiazepine-6-one base, 30–300 nM), a selective muscarinic M_2 receptor antagonist, failed to antagonize acetylcholine-induced reduction of V_{max}, but abolished its increase. It antagonized the shortening of action potential duration, membrane hyperpolarization and decreased the beating rate. McN-A-343 (4-(*m*-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride, 100 and 300 μM), a muscarinic M_1 receptor agonist, reduced V_{max} and prolonged action potential duration, while oxotremorine (100–300 nM), a muscarinic M_2 receptor agonist, evoked reverse effects. These results suggest that acetylcholine exerts a mixed effect on V_{max}, consisting of a reduction and a facilitation, possibly mediated by concurrent activation of muscarinic M_1 and M_2 receptors, respectively, in isolated right atria of mice. © 1998 Elsevier Science B.V.

Keywords: Muscarinic M_1 receptor; V_{max}; Acetylcholine; Atrium, right (Mouse)

1. Introduction

Although major responses of muscarinic receptor agonists in the cardiac muscles are mediated by the activation of muscarinic M_2 receptors (Schimerlik, 1989), muscarinic M_1 receptors also are expressed in the heart (Evans et al., 1985; Gallo et al., 1993; Sharma et al., 1996). Muscarinic M_1 receptors mediate the positive inotropic effects in human atrial trabeculae (Du et al., 1995) as well as the increase in automaticity in canine Purkinje fibers (Rosen et al., 1990). In addition, activation of muscarinic M_1 receptors by carbachol increases the L-type Ca^{2+} current in guinea-pig ventricular cardiocytes, which may be a component of the paradoxical positive inotropism induced by high concentrations of muscarinic receptor agonists (Gallo et al., 1993). A muscarinic M_1 receptor-mediated positive inotropic effect has been suggested as limiting mechanism to prevent excessive suppression of cardiac activity (Pappano, 1991).

However, it is unclear whether muscarinic M_1 receptors are likewise involved in the regulation of cardiac action potentials. In canine Purkinje fibers, pirenzepine, a selective muscarinic M_1 receptor antagonist fails to modify the maximum diastolic potential and activation voltage (Rosen et al., 1990). Acetylcholine causes membrane hyperpolarization and shortening of action potential duration in mammalian atrial tissues by activation of muscarinic M_2 recep-

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tors (Endoh et al., 1985; Freeman, 1979; Koumi et al., 1995). The effect of muscarinic receptor activation on the maximum upstroke velocity of action potentials (V_{max}) is still unclear.

The effects of muscarinic M_1 receptors on cardiac action potentials may provide a basis for better understanding of the pathophysiological significance of these receptors in cardiac function. Abnormalities in the ion fluxes involved in the generation of action potentials are responsible for most cardiac arrhythmias (Katz, 1992). To this end, we have investigated the effects of acetylcholine on action potentials of mouse isolated right atria at concentrations higher than those implicated in the muscarinic M_2 receptor-mediated effects, using a conventional glass microelectrode technique.

2. Materials and methods

2.1. Right atrial preparation

Male ddY mice (30–40 g, 7–9 weeks) were killed by decapitation under light ether anesthesia and the heart was quickly isolated and placed in Krebs–Henseleit solution. The solution was composed of 118.4 mM NaCl, 4.69 mM KCl, 2.0 mM $CaCl_2$, 1.16 mM $MgCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 5.0 mM glucose and gassed with 95% O_2-5% CO_2 (pH = 7.4). Spontaneously beating right atria were separated from ventricle, left atria and other tissues. The atrial preparations were pinned, with the epicardial surface uppermost, to a silicon rubber sheet which covered the bottom of the organ bath (1 ml) and were superfused at a rate of 3 ml/min with Krebs–Henseleit solution. The tissue chamber was maintained at 31 ± 1°C by using a microwarm plate (DC-MP-10 DM, Kitazato, Shizuoka, Japan) to sustain stable recordings of transmembrane potentials accompanied with spontaneous beating.

In some experiments, small pieces ($1 \times 2 \times 4$ mm) of muscle were excised from male the right atria of mice and similarly, from Wistar rats (300–350 g, 9 weeks) and were electrically driven at 4 Hz with pulses of 3 ms duration and 1.5 times threshold diastolic potential.

2.2. Recording of transmembrane potentials

After a 1-h equilibration period, transmembrane potentials were recorded using a standard glass microelectrode filled with 3 M KCl (resistance 8–12 MΩ). A direct current amplifier with high-input impedance (model DPZ-11, Dia medical system, Tokyo, Japan) was used to record membrane potentials. The amplified signals were displayed on a dual beam oscilloscope (VC-9, Nihon Kohden, Tokyo, Japan) and recorded on a cassette data recorder (XR-5000, TEAC, Tokyo, Japan). The recorded signals were then inscribed with a pen recorder (Recti-Horiz 8 K, NEC-Sanei, Tokyo, Japan). The V_{max} (V/s) was estimated by digital differentiation using a programmed FFT (Fast Fourier Transform) analyzer (SM-2701, Iwatsu, Osaka, Japan). The FFT is an accurate representation of V_{max}, because the amplifier output was linear between 10–1000 V/s.

2.3. Experimental protocols

After a stable impalement of a glass electrode was obtained, 7–10 min were allowed to elapse to stabilize the resting membrane potential and the action potential, and control recordings were then done before application of muscarinic agonists (acetylcholine, 1–10 μM; McN-A-343, 30–300 μM; oxotremorine; 100–300 nM). Atria were exposed to these agonists for 10 min and then washed out for another 5–10 min. For comparison, the effects of acetylcholine (5–10 μM) on V_{max} were further tested in electrically driven right atrial preparations from mice and rats. Experiments using muscarinic receptor antagonists were performed with spontaneously beating mouse right atria. In these experiments atropine (3–100 nM), pirenzepine (1–30 nM), AF-DX 116 (30–300 nM) were applied 20–30 min before perfusion of muscarinic receptor agonists and were present throughout the experiments. In some experiments, atria were similarly exposed to propranolol. Reserpine (4 mg/kg, at a bolus) was injected intraperitoneally (i.p.) 4 h before isolation of the atria. This pretreatment is enough to deplete catecholamines from rat heart. (Sjöstrand and Swedin, 1968). Only measurements from continuous microelectrode impalement into one cell per preparation are reported.

The recordings at 0 min indicate the values prior to the application of muscarinic receptor agonists, and in experiments with antagonists, necessarily indicate the values in their presence. To further ensure that only the effects of muscarinic receptor antagonists were observed throughout the entire time course of the experiment, we recorded the action potential parameters for 10 min in the presence of the highest concentrations of antagonists, which were applied 20–30 min prior to the test recordings. Similarly, drug-free control recordings were done.

2.4. Drugs

The following drugs were used: acetylcholine chloride (Daiichi, Tokyo, Japan); McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride); pirenzepine, tetrodoxin and oxotremorine (Sigma, St. Louis, MO, USA); reserpine (Nacalai Tesque, Kyoto, Japan); propranolol hydrochloride (Wako, Osaka, Japan); atropine sulfate (Merck, Darmstadt, Germany) and AF-DX 116 (11({2-[(diethylamino)-methyl]-1-piperidyl}acetyl)-5,11-dihydro-6*H*-pyridol[2,3-b][1,4]benzodiazepine-6-one base, Boehringer Mannheim, Germany). AF-DX 116 was first dissolved in 0.1 N HCl (100 μl) and then diluted with distilled water to make the stock solution. All other

drugs were dissolved in distilled water. Test solutions were prepared by adding appropriate aliquots of the stock to Krebs–Henseleit solution. Adequate measures were taken to protect AF-DX 116 from light.

2.5. Statistics

All the data are means ± S.E.M. The effects of acetylcholine, McN-A-343 and oxotremorine on various action potential parameters were compared with their respective basal values by paired Student's *t*-test. The effects of these agonists in the presence or absence of antagonists were compared by one-way analysis of variance (ANOVA) and Scheffe's multiple-comparison test or by unpaired Student's *t*-test where appropriate. $P < 0.05$ or $P < 0.01$ was adopted as the level of significance.

3. Results

3.1. Acetylcholine-induced reduction of V_{max} in mouse isolated right atria

The effects of acetylcholine on membrane potentials of mouse spontaneously beating right atria are shown in Figs. 1 and 2 and Table 1. After a stable impalement was obtained, acetylcholine was applied by perfusion for 10 min. It produced a concentration dependent reduction of V_{max} at 3–10 μM, while at 1 μM, it did not significantly affect the basal V_{max}. (Fig. 1A). The reduction of V_{max} arose within 1 min, attaining a maximum reduction from 89.4 ± 3.1 V/s to 77.0 ± 4.4 V/s at 3 μM ($n = 12$, $P < 0.05$); from 86.3 ± 3.3 V/s to 70.5 ± 3.0 V/s at 5 μM ($n = 14$, $P < 0.01$) and from 88.8 ± 3.6 V/s to 70.0 ± 4.0 V/s at 10 μM ($n = 12$, $P < 0.01$) within 2–3 min

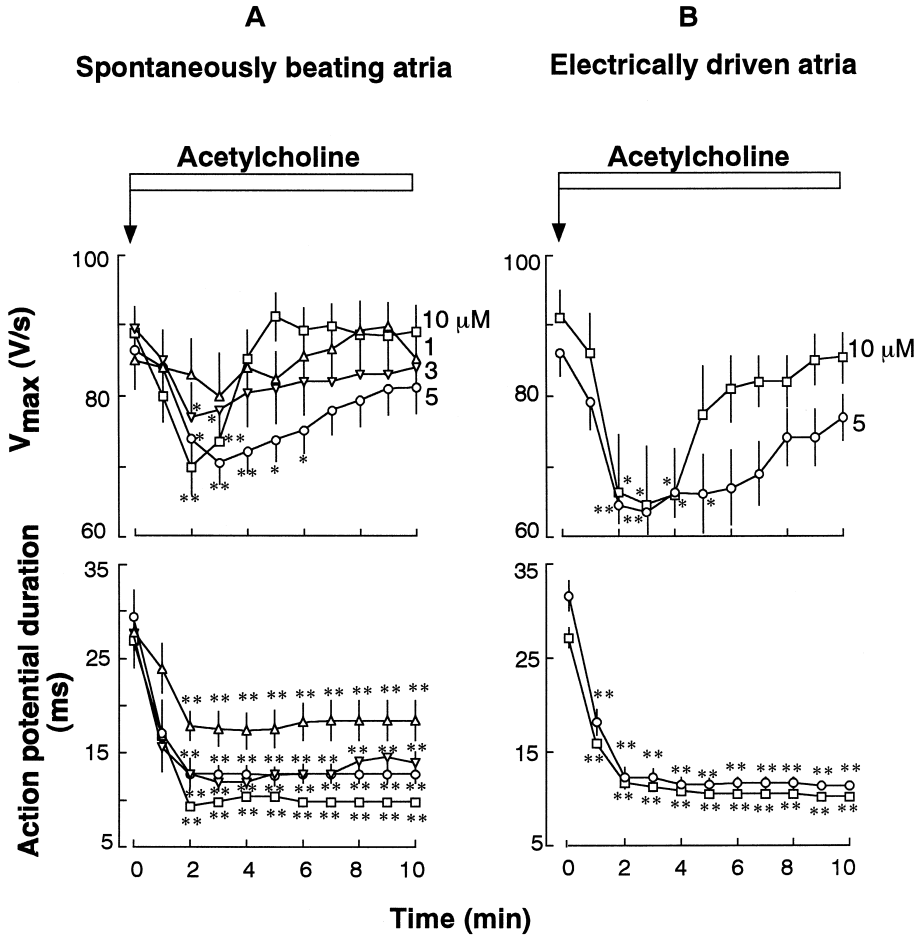

Fig. 1. Reduction of V_{max} (upper panel) and shortening of action potential duration at 90% repolarization (lower panel) by acetylcholine in a concentration- and time-dependent manner in mouse spontaneously beating (A) and electrically driven (B) isolated right atria. After 1-h equilibration of the atria, basal action potentials were recorded for 7–10 min before application of acetylcholine. In A, acetylcholine at 1 (△, $n = 8$), 3 (▽, $n = 12$), 5 (○, $n = 14$) and 10 μM (□, $n = 12$) and in B, at 5 (○, $n = 6$) and 10 μM (□, $n = 4$) was applied by perfusion for 10 min. Values are means ± S.E.M. $* P < 0.05$ and $** P < 0.01$, compared with basal value at 0 min (prior to the application of acetylcholine).

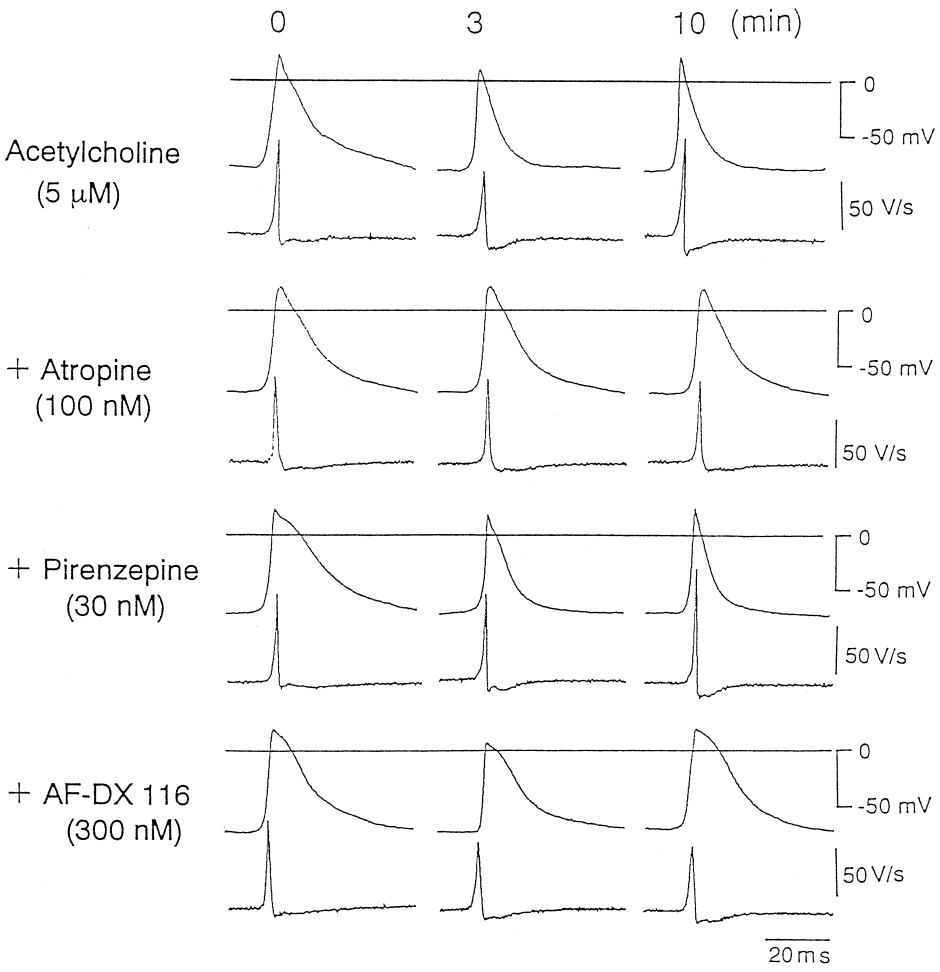

Fig. 2. Typical traces showing the effects of atropine, pirenzepine and AF-DX 116 on the acetylcholine-induced reduction of V_{max} (lower part of each trace), and shortening of action potential duration (upper part of each trace). Traces at 0, 3 and 10 min correspond to the respective response of a representative cell during perfusion of acetylcholine (5 μM) alone and in the presence of atropine (100 nM), pirenzepine (30 nM) or AF-DX 116 (300 nM).

and then gradually returned to its initial levels. Recovery from the reduction was more rapid with 10 μM than with 3 and 5 μM acetylcholine. Action potential amplitude and overshoot potential were likewise reduced by acetylcholine (Fig. 2 and Table 1).

In addition, acetylcholine (1–10 μM) caused a shortening of action potential duration at 90% repolarization (Fig. 1A). Concurrently, it hyperpolarized the resting membrane (Table 1) and exerted a negative chronotropic effect (see below). These changes were evident within 1 min and

Table 1
Acetylcholine-induced hyperpolarization of resting membrane potential and decrease in action potential amplitude and overshoot potential and their antagonism by atropine, AF-DX 116 or pirenzepine

Treatment	Perfusion time (min)	Resting membrane potential (mV)	Action potential amplitude (mV)	Overshoot potential (mV)	*n*
Acetylcholine (5 μM)	0	-72.2 ± 0.4	89.2 ± 0.8	16.8 ± 0.9	10
	3	-75.0 ± 0.6*	82.3 ± 1.4**	7.3 ± 1.8**	
+ Atropine (100 nM)	3	-72.0 ± 0.9†	90.2 ± 0.7††	17.2 ± 1.1††	4
+ Pirenzepine (10 nM)	3	-74.7 ± 1.7	89.8 ± 0.6††	15.1 ± 1.4††	7
+ AF-DX 116 (300 nM)	3	-72.5 ± 1.3†	83.2 ± 1.1	10.2 ± 1.5	4

Values are means ± S.E.M.
* $P < 0.05$, ** $P < 0.01$ compared with corresponding values at 0 min (prior to application of acetylcholine).
† $P < 0.05$, †† $P < 0.01$ compared with corresponding effects of acetylcholine alone.

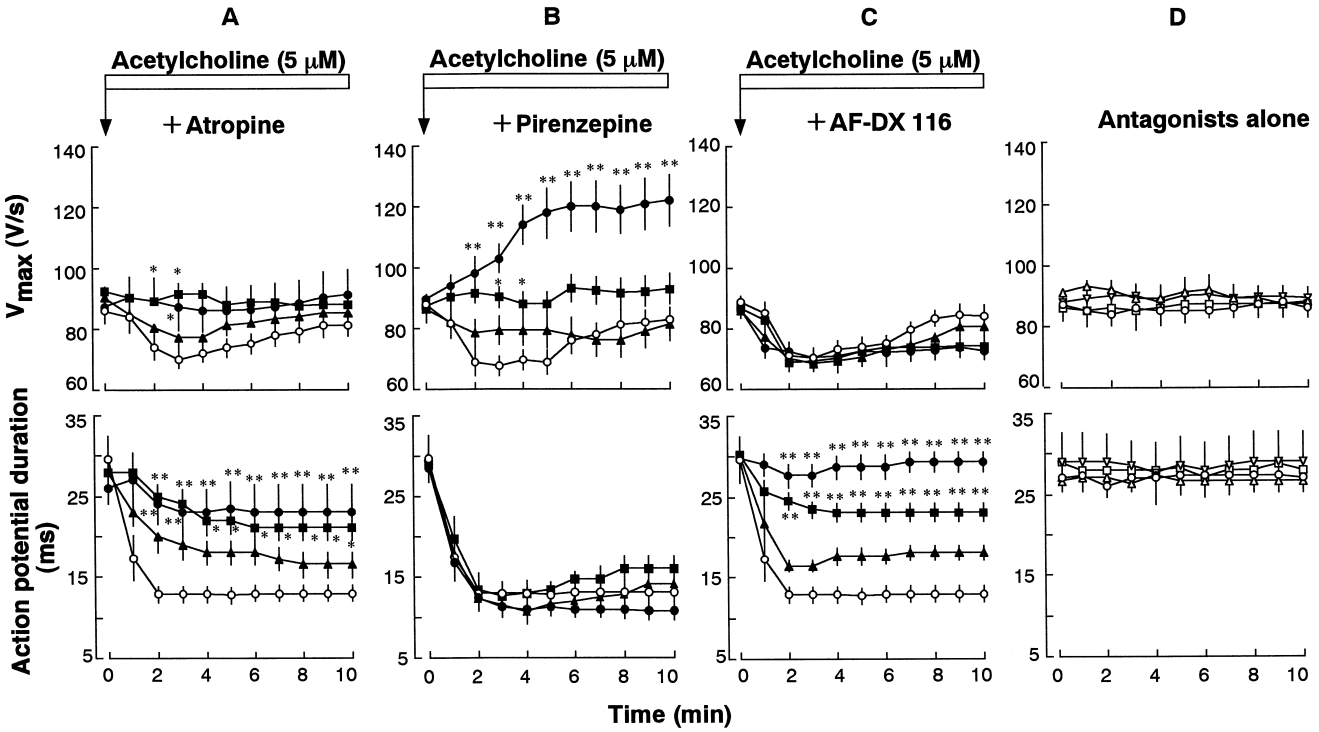

Fig. 3. Effects of atropine (A), pirenzepine (B) or AF-DX 116 (C) on acetylcholine-induced reduction of V_{max} and shortening of action potential duration at 90% repolarization. In A, the atrial preparations were pretreated with 3 (▲, $n = 4$), 30 (■, $n = 5$) or 100 nM (●, $n = 4$) of atropine for 20–30 min, which was present during the 10-min perfusion of acetylcholine (5 μM). Similar pretreatment was done in B with pirenzepine 1 (▲, $n = 7$), 10 (■, $n = 7$) and 30 nM (●, $n = 4$). In C, experiments were done with 30 (▲, $n = 5$), 100 (■, $n = 6$) and 300 nM (●, $n = 4$) AF-DX 116. In D, effects of atropine (100 nM, △, $n = 3$), pirenzepine (30 nM, ▽, $n = 5$) or AF-DX 116 (300 nM, □, $n = 4$), all alone, were determined for 10 min after 20 to 30-min pretreatment and were compared with drug-free control (○, $n = 3$). Values are means ± S.E.M. $* P < 0.05$ and $** P < 0.01$, compared with the effect of acetylcholine (5 μM, ○, $n = 7$–10).

became constant within 2–3 min of perfusion and persisted throughout the time course of the experiment. All these changes subsided after 5–10 min washout (data not shown).

The V_{max} in mouse isolated right atria was significantly reduced by tetrodotoxin (1 μM), a Na^+ channel blocker. In addition, the V_{max} traces were widened, with concomitant reduction of action potential amplitude and disappearance of overshoot potential (data not shown).

Acetylcholine-induced reduction of V_{max} and other changes in action potentials were independent of catecholamine release, because reserpine pretreatment (4 mg/kg, i.p., 4 h) or propranolol (0.3 μM) failed to alter these effects (data not shown).

In electrically driven atrial preparations, acetylcholine (5 and 10 μM) reduced V_{max}. There followed an increase and shortened action potential duration in a pattern similar to that seen in spontaneously beating preparations (Fig. 1B).

For comparison, the effect of acetylcholine was tested in electrically driven right atrial preparations from rats. Acetylcholine (10 μM) significantly reduced the V_{max} from a baseline value of 95.0 ± 3.0 V/s to a value of 71.3 ± 6.2 V/s ($n = 3$, $P < 0.05$) within 1–2 min after application, which was subsequently increased to the previous level within 5 min (data not shown).

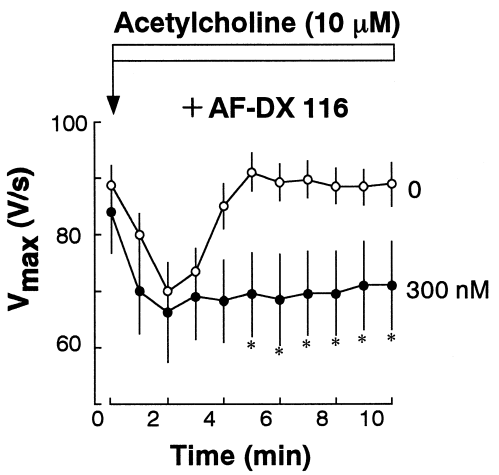

Fig. 4. AF-DX 116 did not antagonize the acetylcholine-induced reduction of V_{max}, but sustained it by abolishing the recovery phase. The atrial preparations were pretreated with 300 nM AF-DX 116 (●, $n = 5$) for 20–30 min, which also was present during the 10-min perfusion of acetylcholine. Values are means ± S.E.M. $* P < 0.05$, compared with the effect of acetylcholine (10 μM, ○, $n = 10$).

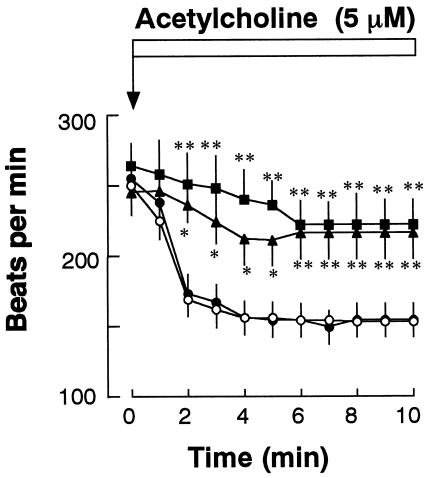

Fig. 5. Acetylcholine-induced decrease in the rate of spontaneous beating and its antagonism by atropine and AF-DX 116, but not by pirenzepine. The atrial preparations were pretreated with atropine (100 nM, ■, $n = 4$), pirenzepine (30 nM, ●, $n = 4$) or AF-DX 116 (300 nM, ▲, $n = 4$) for 20–30 min, which were present during the 10-min perfusion of acetylcholine (5 μM). Values are means ± S.E.M. $*\ P < 0.05$ and $**\ P < 0.01$, compared with the effect of acetylcholine (5 μM, ○, $n = 7$).

3.2. Antagonism of acetylcholine-induced reduction of V_{max}, and its acceleration by muscarinic M_1 receptor antagonist in spontaneously beating atria

To clarify the mode of acetylcholine-induced reduction of V_{max}, action potential amplitude and overshoot potential, different muscarinic receptor antagonists were tested (Figs. 2 and 3 and Table 1).

Atropine (3–100 nM), a non-selective muscarinic receptor antagonist, antagonized acetylcholine-induced reduction of V_{max} and shortening of action potential duration at 90% repolarization (Figs. 2 and 3A). The membrane hyperpolarization, reduction of action potential amplitude and overshoot potential (Table 1), and the negative chronotropic effect (see below) were likewise antagonized.

Pirenzepine (10 and 30 nM), a selective muscarinic M_1 receptor antagonist, selectively antagonized the acetylcholine-induced reduction of V_{max}, action potential amplitude and overshoot potential. It antagonized the acetylcholine-induced reduction of V_{max} after 3 min of perfusion, by increasing V_{max} from 67.5 ± 3.5 V/s ($n = 10$) to

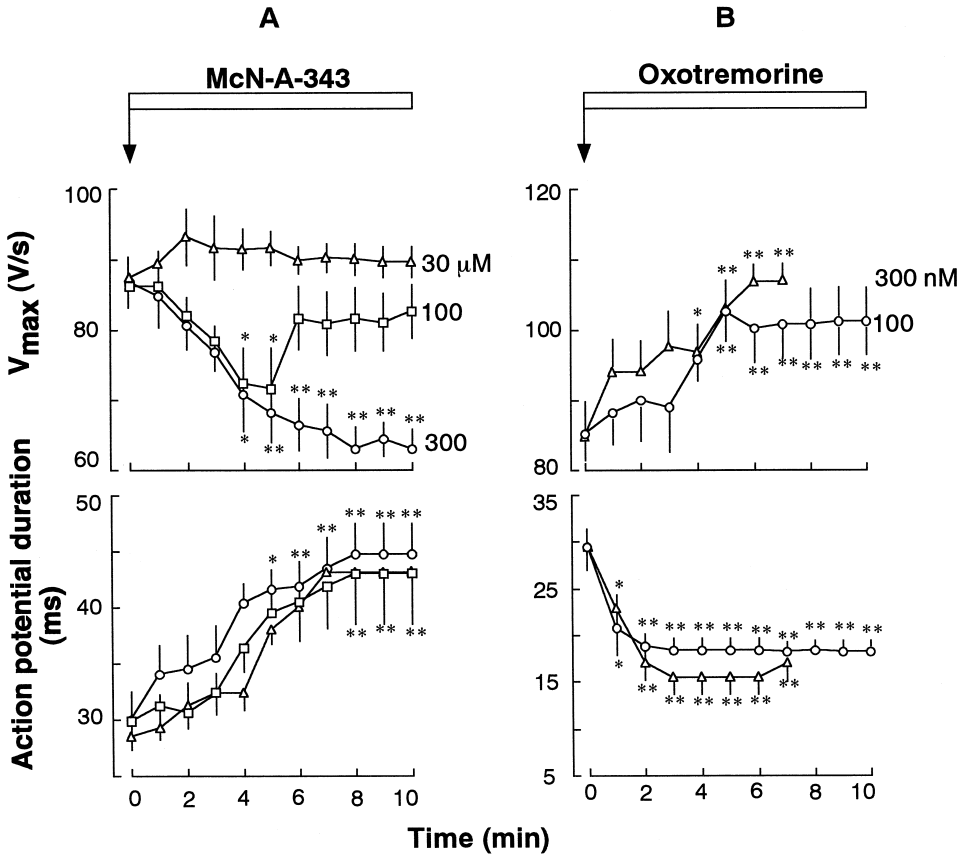

Fig. 6. Reduction of V_{max} and prolongation of action potential duration at 90% repolarization by McN-A-343 (A) and acceleration of V_{max} and shortening of action potential duration at 90% repolarization by oxotremorine (B). After 1-h equilibration of the atria, basal action potentials were recorded for 7–10 min and then McN-A-343, 30 (△, $n = 4$), 100 (□, $n = 5$) and 300 μM (○, $n = 5$) was applied by perfusion for 10 min. Similarly, experiments were done with 100 (○, $n = 4$) and 300 nM (△, $n = 4$) of oxotremorine. Values are means ± S.E.M. $*\ P < 0.05$ and $**\ P < 0.01$, compared with the basal value at 0 min (prior to the application of McN-A-343 or oxotremorine).

90.2 ± 3.4 V/s ($n = 7$, $P < 0.05$) and 103 ± 4.5 V/s ($n = 4$, $P < 0.01$) at 10 and 30 nM respectively. In addition, with pirenzepine (30 nM) there was an immediate and linear acceleration of V_{max} (Figs. 2 and 3B) which was completely abolished in the simultaneous presence of AF-DX 116 (300 nM), a muscarinic M_2 receptor antagonist (data not shown). Pirenzepine (30 nM) accelerated the V_{max} value from 80.3 ± 3.9 V/s (acetylcholine alone, $n = 10$) to 122 ± 8.4 V/s ($n = 4$, $P < 0.01$) after 10 min of acetylcholine perfusion. Shortening of action potential duration at 90% repolarization, membrane hyperpolarization and the negative chronotropic effect of acetylcholine remained unaffected at the same concentrations of pirenzepine.

As shown in Figs. 2 and 3C and Table 1, AF-DX 116 (30–300 nM) did not antagonize the acetylcholine-induced reduction of V_{max}, action potential amplitude and overshoot potential when compared with acetylcholine alone effects. AF-DX 116 (300 nM) sustained the acetylcholine (10 μM)-induced reduction of V_{max}, and abolished its facilitation (Fig. 4). AF-DX 116 antagonized the acetylcholine (5 μM)-induced shortening of action potential duration at 90% repolarization (Fig. 3C), the membrane hyperpolarization (Table 1) and the negative chronotropic effect (Fig. 5).

The muscarinic antagonists used did not affect the basal V_{max}, action potential duration (Fig. 3D) and other action potential parameters (data not shown). Therefore, the above results suggest that acetylcholine-induced reduction of V_{max} and its increase involve muscarinic M_1 and M_2 receptors respectively.

3.3. Reduction of V_{max} by McN-A-343, a selective muscarinic M_1 receptor agonist, and acceleration by oxotremorine, a muscarinic M_2 receptor agonist

To further substantiate the involvement of muscarinic receptor subtypes in acetylcholine effects on the V_{max} and associated parameters we investigated the effects of McN-A-343, a muscarinic M_1 receptor agonist, and oxotremorine, a muscarinic M_2 receptor agonist.

As shown in Fig. 6A, McN-A-343 at 30 μM, did not affect V_{max}, while at 100 and 300 μM of it concentration

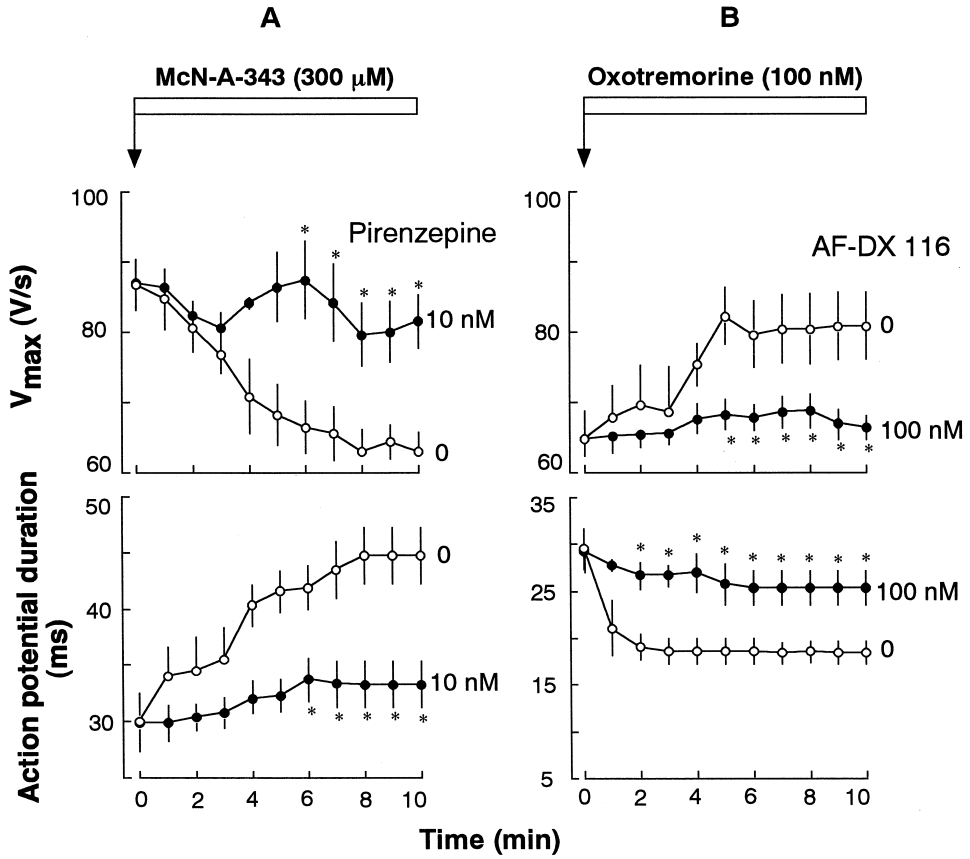

Fig. 7. Antagonism, by pirenzepine (A) of the McN-A-343-induced reduction of V_{max} and prolongation of action potential duration at 90% repolarization and antagonism by AF-DX 116 (B) of the oxotremorine-induced acceleration of V_{max} and shortening of action potential duration at 90% repolarization. The atrial preparations were pretreated with 10 nM pirenzepine (●, left panel, $n = 4$) and 100 nM AF-DX 116 (●, right panel, $n = 4$) for 20–30 min, which were present during the 10-min exposure to McN-A-343 and oxotremorine, respectively. Values are means ± S.E.M. $* P < 0.05$, compared with the effect of McN-A-343 (300 μM, ○, A) or oxotremorine (100 nM, ○, B).

Table 2
McN-A-343- and oxotremorine-induced changes in resting membrane potential, action potential amplitude and overshoot potential and their antagonism by pirenzepine and AF-DX 116 respectively

Treatment	Perfusion time (min)	Resting membrane potential (mV)	Action potential amplitude (mV)	Overshoot potential (mV)	*n*
McN-A-343	0	-73.6 ± 0.8	89.8 ± 1.6	16.2 ± 1.3	4
	8	-71.9 ± 1.0	84.0 ± 2.1**	12.2 ± 2.0*	
+ Pirenzepine (10 nM)	8	-72.2 ± 0.1	89.0 ± 0.2††	16.4 ± 0.1†	4
Oxotremorine (100 nM)	0	-73.5 ± 1.4	88.0 ± 1.5	18.6 ± 2.6	6
	8	-76.1 ± 0.8**	97.0 ± 3.4**	26.2 ± 2.0**	
+ AF-DX 116 (100 nM)	8	-73.0 ± 0.4††	88.7 ± 0.7††	15.7 ± 0.4††	4

All values are means ± S.E.M.
* $P < 0.05$, ** $P < 0.01$ compared with corresponding values at 0 min (prior to the application of McN-A-343 or oxotremorine).
† $P < 0.05$, †† $P < 0.01$ compared with corresponding effects of McN-A-343 or oxotremorine alone.

dependently reduced V_{max}. The concentrations of McN-A-343 used in this study were similar to those used to activate muscarinic M_1 receptors in human atrial trabeculae (Du et al., 1995) and guinea pig ventricular myocytes (Matsumoto and Pappano, 1991). Unlike with acetylcholine, there was no biphasic response at 300 μM of McN-A-343. The reducing effect was evident at 4–5 min then attained a constant level at 7–8 min. After 8 min of perfusion McN-A-343 (300 μM) reduced V_{max} to 63.0 ± 3.2 V/s from the basal level of 86.8 ± 3.6 V/s ($n = 5$, $P < 0.01$). Concomitantly, action potential amplitude and overshoot potential were likewise reduced (Table 2). McN-A-343 did not cause any hyperpolarization, but tended to depolarize the tissue at a high concentration (300 μM). Furthermore, it prolonged action potential duration at 90% repolarization concentration dependently (Fig. 6A). The maximum prolongation by McN-A-343 (300 μM) occurred at 8 min of perfusion from 30 ± 2.6 ms to 45 ± 2.5 ms ($n = 5$, $P < 0.01$). All the McN-A-343-induced effects were significantly antagonized by pirenzepine (10 nM) (Fig. 7A and Table 2).

Oxotremorine (100–300 nM) significantly accelerated V_{max}, action potential amplitude and overshoot potential from their respective basal levels. Similar to acetylcholine, oxotremorine further caused shortening of action potential duration at 90% repolarization and membrane hyperpolarization (Fig. 6B and Table 2). At 300 nM, oxotremorine stopped, and eliminated further spontaneous action potentials after 7 min of perfusion in all preparations. AF-DX 116 (100 nM) blocked the effects of oxotremorine (Fig. 7B and Table 2).

4. Discussion

This is the first report that acetylcholine reduces V_{max}, followed by its increase, in the isolated spontaneously beating right atria of mice. As similar results were obtained in electrically driven atria, the changes in V_{max} are not secondary to changes in beating rate or threshold potential. Furthermore, acetylcholine-induced reduction of V_{max}, being affected by neither prior reserpinization nor propranolol, is independent of endogenous catecholamine release. Acetylcholine increases the V_{max} in guinea-pig (Freeman, 1979) and human atria (Jakob et al., 1989). However, in isolated right atria of rat we found a reduction, followed by an increase in V_{max} by acetylcholine.

The muscarinic M_2 receptors mediate the major responses to muscarinic receptor agonists in cardiac muscles (Schimerlik, 1989). Several lines of evidence suggest the molecular and functional entity of muscarinic M_1 receptors in mammalian heart (Kubo et al., 1986; Gallo et al., 1993; Du et al., 1995; Sharma et al., 1996). We now provide evidence that muscarinic M_1 receptors mediate the acetylcholine-induced reduction of V_{max} while muscarinic M_2 receptors mediate its increase.

Pirenzepine, a selective muscarinic M_1 receptor antagonist (Hammer et al., 1980; Eglen and Watson, 1996; Sun et al., 1996), antagonized the acetylcholine (5 μM)-induced reduction in V_{max} at 10 nM and immediately accelerated the V_{max} at 30 nM. The concentrations of pirenzepine used are consistent with that shown specifically to block the cardiac muscarinic M_1 receptors (Gallo et al., 1993; Sharma et al., 1996) and are approximately 30 times lower than the pirenzepine dissociation constant for muscarinic M_3 receptors (Sun et al., 1996). Therefore, we consider that muscarinic M_1 receptors are involved in the acetylcholine-induced reduction of V_{max}. AF-DX 116, a preferentially selective muscarinic M_2 receptor antagonist (Giachetti et al., 1986; Hulme et al., 1990), did not antagonize the acetylcholine-induced reduction of V_{max}, but sustained it by abolishing its facilitation. This result suggests that the facilitation of V_{max} by acetylcholine involves muscarinic M_2 receptors. AF-DX 116, at concentrations similar to that we used in the present study or even higher, blocks muscarinic M_2 receptor-mediated effects, but not the M_1 receptor-mediated cardiac positive chronotropic (Rosen et al., 1990; Sun et al., 1994) and inotropic (Du et al., 1995) effects. A weak discrimination by this antagonist between muscarinic M_1 (rabbit vas deferens) and M_2 (guinea-pig atria) receptors as shown previously (Dörje et al., 1990) may not be implied for cardiac M_1 and M_2 receptors.

The results with acetylcholine and AF-DX 116 suggest that the reduction and facilitation in V_{max} apparently occur

in a sequential fashion. However, the immediate and linear acceleration of V_{max} caused by the mixture of acetylcholine (5 μM) and pirenzepine (30 nM), and its abolition by AF-DX 116 (300 nM) suggest that the acetylcholine-induced reduction and the facilitation in V_{max} are mixed effects, possibly mediated by the concurrent activation of muscarinic M_1 and M_2 receptors respectively. These two phenomena seem to have a balancing action on each other, and it is difficult to ascertain which of these effects is the most sensitive to acetylcholine. Thus, it is possible that the blockade of muscarinic M_1 receptors by pirenzepine unmasks the M_2 receptor-mediated facilitation and thereby accelerates V_{max}. It could be argued that an endogenous release of acetylcholine, possibly caused by pirenzepine at 30 nM preferentially acting on the muscarinic M_2 receptors, accelerates the V_{max}. Such a possibility is unlikely, because pirenzepine alone affected neither V_{max} and other action potential parameters nor the rate of beating. Moreover, if, in the presence of exogenous acetylcholine, pirenzepine could evoke the release of endogenous acetylcholine, in turn, it should concurrently accelerate other acetylcholine effects. In fact, we did not observe such phenomena. Atropine blocked the reduction and caused no acceleration possibly because of its non-selective blocking of both muscarinic M_1 and M_2 receptors.

McN-A-343, a preferentially selective muscarinic M_1 receptor agonist (Hammer and Giachetti, 1982; Eltze, 1988; Hu and El-Fakahany, 1990; Sun et al., 1994), reduced V_{max} and prolonged action potential duration, effects which were antagonized by pirenzepine (10 nM), indicating muscarinic M_1 receptor-mediating actions. In guinea-pig heart, McN-A-343 causes acetylcholine-like effects (e.g., shortening of action potential duration and membrane hyperpolarization) (Pappano and Rembish, 1971). It is unclear whether McN-A-343 causes acetylcholine release in cardiac tissues, although there is evidence for this in pelvic ganglion of the rat (Somogyi and De Groat, 1993). It facilitates noradrenaline release in mouse atria by activation of muscarinic M_1 receptors (Costa and Majewski, 1991). Oxotremorine has preferential selectivity for muscarinic M_2 receptors (Gupta et al., 1994; Emala et al., 1995; Sun et al., 1996). The increase in V_{max} by oxotremorine and its antagonism by AF-DX 116 in our present study suggest the involvement of muscarinic M_2 receptors, which may be explained as an indirect effect of hyperpolarization (Jakob et al., 1989).

The post receptor mechanisms of muscarinic M_1 and M_2 receptor-mediated effects on V_{max} are not clear. Muscarinic M_1 and M_2 receptors are coupled to the activation of phosphoinositide hydrolysis (Hosey, 1992; Gallo et al., 1993) and G_i-protein (Schimerlik, 1989) respectively.

The opposing actions of muscarinic M_1 and M_2 receptors on V_{max} may conform to the yin–yang hypothesis, analogously to those of acetylcholine and β-adrenoceptor agonists on cyclic AMP synthesis (Goldberg and Haddox, 1977). The stimulatory actions of muscarinic M_1 receptors on cardiac contractility have been suggested to serve as compensatory mechanism against the inhibitory actions of muscarinic M_2 receptors (Pappano, 1991; Gallo et al., 1993; Sharma et al., 1996). The V_{max} is an indirect measurement of the peak Na^+ current (Sheets et al., 1988) and its inhibition is considered as an antiarrhythmic profile (Grant et al., 1984). Thus, the muscarinic M_1 receptor-mediated reduction of V_{max} may represent a potential antiarrhythmic activity.

In conclusion, acetylcholine exerts a mixed effect on V_{max}, consisting of a reduction and a facilitation, possibly mediated by the concurrent activation of muscarinic M_1 and M_2 receptors, respectively, in isolated right atria of mice.

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